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14. ABSTRACT: The goals of this application are to develop methods to non-invasively differentiate fast and slow growing prostate tumors and also develop methods to evaluate response to anti-angiogenic agents. Validation of the results will be based on tumor growth, metastases, and microvessel density measurement (anti-angiogenic studies). To date, we have focused on optimizing the pulse sequences necessary for lactate detection, synthesizing a macromolecular contrast agent and optimizing its use. These goals have been more difficult to achieve but we are now able to localize lactate within a voxel of the tumor with quantitation, and have finished synthesizing the macromolecular contrast agent and been using it in studies					
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Introduction

The primary goal of this study is to determine whether non-invasive magnetic resonance (MR) techniques can distinguish between slow and rapidly growing and metastatic prostate tumors. This is particularly important in prostate cancer where 30% of men over the age of 50 have prostate cancer at autopsy but only 10% of men develop prostate cancer. Reliable methods do not exist to determine which cancers are aggressive and need to be treated vs. patients who could undergo watchful waiting. A second goal is to determine if anti-angiogenesis agents can be used as chronic low toxicity therapy for “newly diagnosed” tumors and as adjuvant to “curative” therapies and if MR techniques can be used as an early or *a priori* marker of tumor response. We propose that non invasive measurements of tumor vascular volume, permeability, choline and lactate will predict tumor aggressiveness (growth rate, tendency to metastasize). The hypothesis is based on data which indicates that tumor growth rates and metastases are related to angiogenesis which can be detected by dynamic contrast enhanced magnetic resonance imaging (DCE-MRI).

In addition, we propose to determine whether chronic anti-angiogenic therapy can be used in small “newly diagnosed” tumors and as an adjuvant to radiation. It will also be determined whether MRSI and DCE-MRI can predict response to anti-angiogenic therapy. We will also study the effect of anti-angiogenic therapy in small “newly diagnosed” tumors and also as an adjuvant post radiation to determine if it delays tumor growth and metastases. Tumor doubling times and number of metastatic lesions in the lung will be the biological outcome measures. Developing chronic treatment modalities that delay (or obviate) the need for radical therapy will enhance treatment of prostate cancer and possibly quality of life. Similarly, developing a treatment that enhances response to radiation could potentially enhance the cure rate or delay recurrence which might alter subsequent therapy. The techniques applied here are available or readily implemented on clinical scanners (choline and lactate detection; DCE-MRI) so the research is highly translational.

Body

At the time of the previous report (9 months ago), we had finally succeeded in obtaining lactate data in phantoms. We had not expected any problems with this at the time of submission of the original application, but the use of a new instrument and major changes in personnel, required us to basically start from the beginning. Over the last year, we have made significant technical progress such that we are now able to obtain quantitative localized data serially on tumor bearing rats and have worked out the technical problems, as can readily be appreciated from the data below. Furthermore, at the time of writing last year's report (9 months ago), we had not successfully been able to grow the Dunning H model (slow growing minimally invasive cancer). This has also been resolved. On the disappointing side, we have only now (after several false starts) begun collecting data.

1. Quantitation. There are two general methods of trying to obtain absolute concentrations of metabolites from NMR data. These include the "substitution" method (1) and calibrating against an external standard (2). We have used the latter technique many times (3-5), however, in this situation, because of the geometry of the coil, the substitution method was more appropriate. This method requires careful measurements of the relative power each time one acquires data and comparing the power required for different pulses when the subject (experimental rat) is in place, vs that required when a phantom (whose quantitation is accurately known) is in place. We initially tried the external method and realized it would not work and switched to the substitution method. However, to accomplish this, we needed to be able to excite the slice of interest off the center axis of the magnet.

Slice-selective SelMQC:

Conventional SelMQC sequence is used for detecting lactate (Lac) signals by completely suppressing the co-resonating lipid (Lip) resonances as well as water resonance in a single scan. This sequence employs all frequency selective pulses to prepare multiple quantum coherences and gradients were applied during the pulse sequence to choose the desired pathway of detection. We previously implemented slice selective pulses on the Bruker 4.7T Bruker scanner. In this work, a slice select gradient was added to the conventional non-localized SelMQC sequence by replacing the first frequency selective pulse in the sequence by broad bandwidth pulse so that all the spins within the slice are uniformly excited to achieve the slice selection. In our present experiment three lobe sinc pulses have been used as slice-select pulse. For reduced slice thicknesses, the width of the slice is controlled by gradient strength. We used 16 x 16 phase encoding steps resulting in CSI matrix size is 16 x 16 x 256 and FOV is 40mm leading to 2.5 x 2.5 mm² in plane resolution. We can choose slices in any direction by choosing gradient directions. From the data in Fig.1, the 5mm sagittal 2D CSI was overlaid on a T2-weighted MR image and the lipid and water suppression is still effective similar to the regular selMQC without slice selection. Minimal peaks are seen in the area containing lipid and signal is largely confined to the lactate area.

In animal tumors, it is important to be able to acquire the slices off center. We implemented this modification using a two compartment model phantom with 50 mM Lactate solution and Crisco

shortening side by side to test our acquisition of off-centered slices. Fig. 2 shows a coronal T2-weighted image of the phantom, with the lactate area having a higher signal due to the short T2 of the shortening. Fig.3 represents the net lactate signal (in either lactate or Crisco phantom) acquired sequentially from a 5mm slice centered around the positions indicated on the plot shown in Fig. 3. To confirm the spatial distribution using off-centered slice select pulses, we obtained sagittal 2D CSI slices with 5mm slice thickness from lactate side showing uniform lactate distribution and absence of lactate signal from fat side (Fig. 4).

We have to date studied 2 tumor bearing rats, each 4 times. The data are shown in Fig. 5. In addition, we have acquired DCE-MRI data after Gd-DTPA and Gd-DTPA-albumin.

At the same time, we have also been growing the Dunning H tumor, which we had previously been unsuccessful. To date, we have studied four rats with tumors ranging from 105 to 305 mm³, two rats with tumors in the range 36 to 89 mm³, one rat with a very small tumor, and two others we are watching. Thus we have finally been successful in establishing the slow growing Dunning H model in the laboratory.

Data Presentation

A. Data acquisition:

MR lactate determination and perfusion studies were conducted at four tumor sizes: (I: 300-600mm³, II: 700-1000 mm³, III: 1200-1600 mm³ and IV: 1700-2000 mm³) in Copenhagen rats. Experiments were performed on a Bruker 4.7 T, 40-cm-bore animal scanner. A home-made 2 turn volume coil with 25 mm diameter was used as a transmit-receive RF coil. Frequency selective 15 ms single-lobe Sinc pulses were employed for Sel-MQC (SElective Multiple Quantum Coherence) editing. The ZQ (zero quantum) →DQ (double quantum) coherence transfer pathway was selected in Sel-MQC experiments using a phase cycling gradient combination of $g_1:g_2:g_3 = 0:-1:2$ with duration $\delta_1 = \delta_2 = 2$ ms, $\delta_3 = 4$ ms, and amplitude of 24 G/cm. 512 data points were collected with 8 averages, TR=2 s and spectral width of 2500Hz. A matrix size of 16×16, FOV = 40 mm (2.5 x 2.5 mm in plane resolution) was used. Two-dimensional chemical shift imaging lactate map is generated by selecting a 5 mm slice in the tumor region. The 2D CSI lactate map is coregistered with T2-weighted image of 5 mm slice thickness.

B. Processing:

Visualization of the overlaid 2D CSI lactate map with T2-weighted image (Fig. 5) was done using 3DCSI software.

C. Quantification:

- 1) Prior to using the window functions, FIDs (Free Induction Decay) from each voxel were exported as text files for further processing and quantification using JMRUI.

- 2) The exported text file (voxel FID) was opened in JMRUI software and all the acquisition parameters were input into the program.
- 3) Lorentzian/Gaussian window functions were applied to Fourier transform the FID to signal (Fig.6).
- 4) The lactate signal was integrated to get the area under the peak, which was used for quantification along with coil loading parameters and reference lactate signal intensity using the equation:

Test lactate concentration =

$$\frac{(\text{Test voxel area} / \text{Reference voxel area}) * \text{concentration of reference} * (\text{reference voltage} / \text{test voltage})}{(\text{NS}_{\text{test}} / \text{NS}_{\text{ref}})}$$

where NS_{test} and NS_{ref} are the number of excitations for the test object and the reference phantom

D. Results

Figure 5 shows a 2D-CSI image overlaid on the anatomic image of the same rat and shows the lactate peak clearly visible, and figure 6 shows the fitting of the data which is noted to be excellent based on the lack of a residual signal that can be recognized.. Figs. 7,8 show the preliminary data correlating tumor volume with k_{ep} and lactate. More data are being accumulated to provide statistically valid conclusions.

KEY RESEARCH ACCOMPLISHMENTS:

1. Pulse sequence implemented to measure lactate, both on center slice of test object and off center
2. Implementation of quantitation methods

REPORTABLE OUTCOMES:

Abstract to be submitted for upcoming Society of Molecular Imaging meeting

CONCLUSION:

We have in essence completed all the technical hurdles to accomplish the proposed research. These technical details required far more effort than expected but were necessary to overcome. These results will be applicable to many other research efforts ongoing both in the principal investigator's laboratory and others.

REFERENCES:

1. [Arslanoglu A](#), [Bonekamp D](#), [Barker PB](#), [Horska A](#). Quantitative proton MR spectroscopic imaging of the mesial temporal lobe. [J Magn Reson Imaging](#). 2004 Nov;20(5):772-8
2. Murphy-Boesch, J.; Jiang, H.; Stoyanova, R.; Brown, T.R. Quantification of Phosphorus Metabolites from Chemical Shift Imaging Spectra with Corrections for Point Spread Effects and B₁ Inhomogeneity. *Magn. Reson. Med.* 39:429-438; 1998
3. Zakian, K.L., D'Angelica, M.D., Matei, C., Yoo, H-H., Ercolani, M., Fong, Y., Koutcher, JA A Quantitative Assessment of Liver Metabolites during Jaundice Using Three Dimensional Phosphorus Chemical Shift Imaging. *Magn. Reson. Imag.* Vol 18. pp. 181-187, 2000.
4. [Zakian KL](#), [Koutcher JA](#), [Malhotra S](#), [Thaler H](#), [Jarnagin W](#), [Schwartz L](#), [Fong Y](#) Liver regeneration in humans is characterized by significant changes in cellular phosphorus metabolism: assessment using proton-decoupled ³¹P-magnetic resonance spectroscopic imaging. [Magn Reson Med](#). 2005 Aug;54(2):264-71
5. [Kooby DA](#), [Zakian KL](#), [Challa SN](#), [Matei C](#), [Petrowsky H](#), [Yoo HH](#), [Koutcher JA](#), [Fong Y](#). Use of phosphorous-31 nuclear magnetic resonance spectroscopy to determine safe timing of chemotherapy after hepatic resection. [Cancer Res](#). 2000 Jul 15;60(14):3800-6

APPENDICES: None

SUPPORTING DATA:

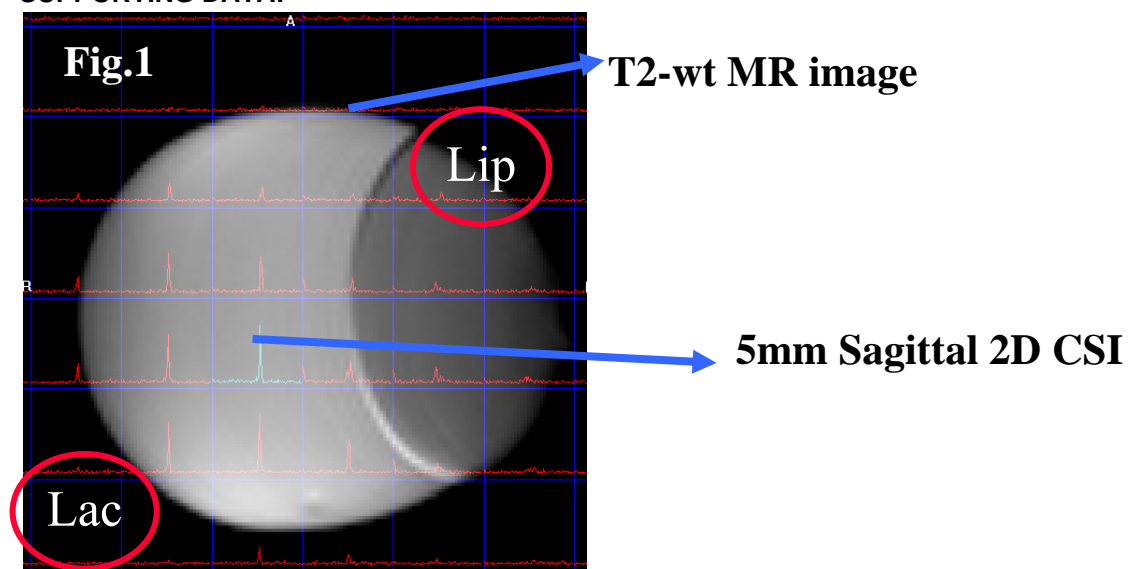


Fig. 1. Images of a phantom containing lipid (Lip) and lactate (Lac) showing selective detection of lactate with almost no signal from lipid

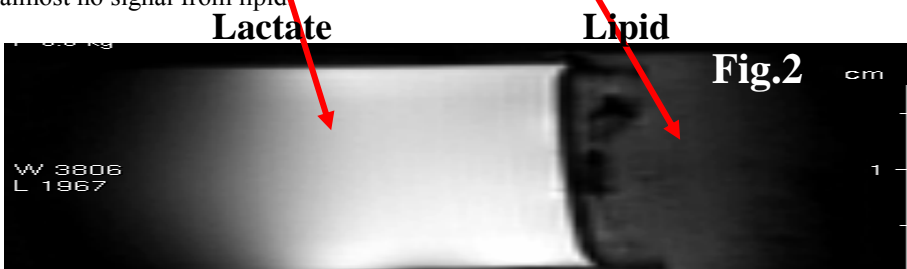


Fig. 2. Coronal image of a 50 mM lactate and Crisco (lipid) phantom demonstrating for testing Sel-MQC off center axis. The lactate signal is stronger due to the short T2 of the shortening. Image of a phantom

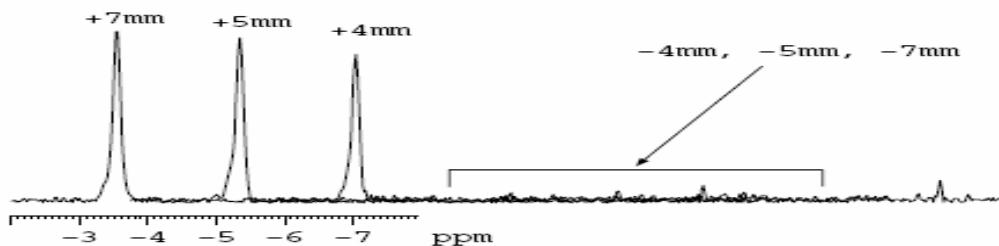


Fig.3. Net lactate signal (in either lactate or Crisco phantom) acquired sequentially from 5mm slices centered around the positions indicated

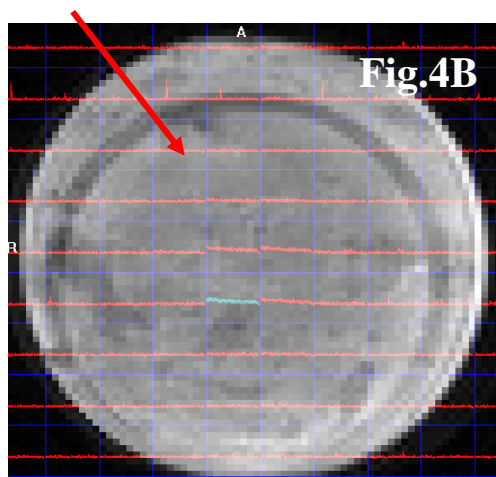
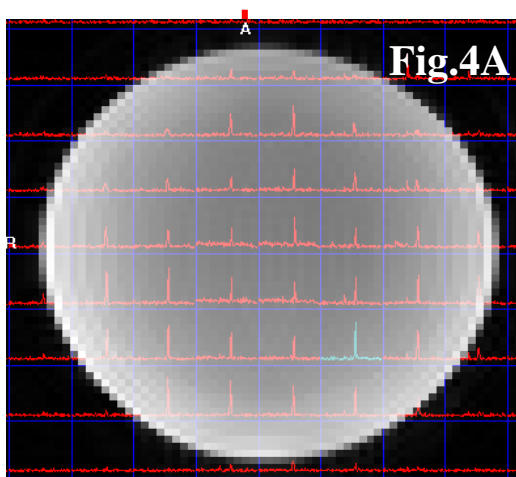


Fig. 4. 2D CSI slices with 5mm slice thickness from lactate sagittal side (4A) showing uniform lactate distribution and absence of lactate signal from fat side (Fig. 4B).

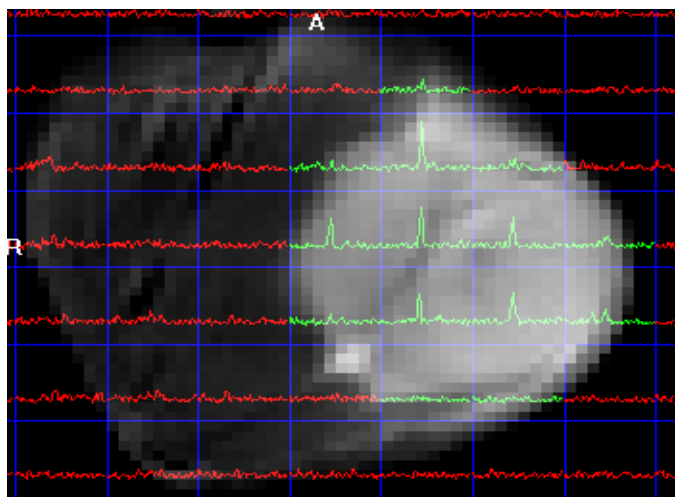


Fig. 5 2D-CSI slice taken from a Dunning R3327-AT fast growing tumor. Note presence of lactate and note that regions to the left of the tumor with lower lactate appear necrotic.

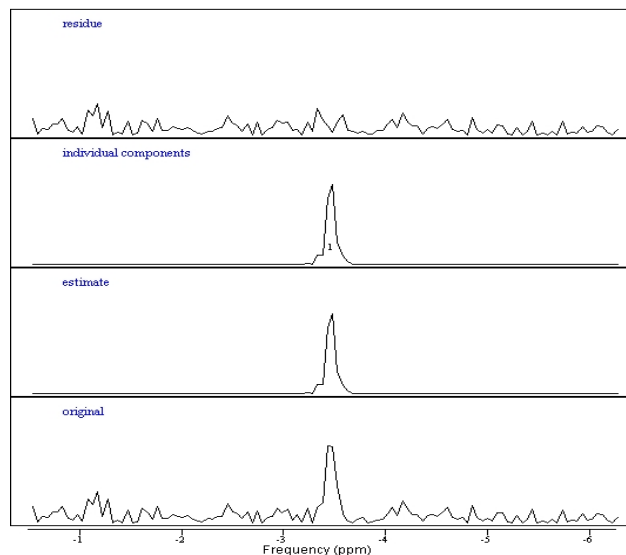


Fig. 6 Curve fitting of a peak from a lactate voxel. The original signal is on the bottom, followed by the estimate of the peak fit with the individual components. The top spectrum shows the excellence of the fit since no residual signal is seen, only noise

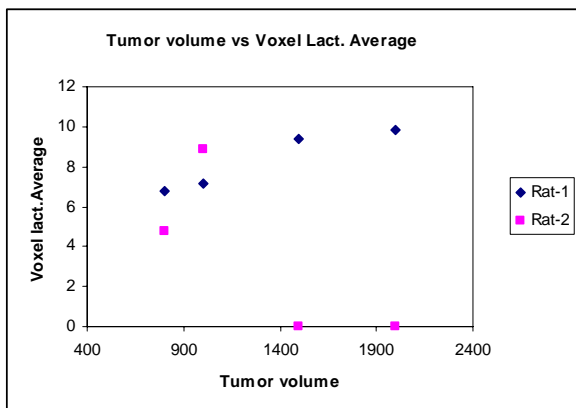


Fig. 7. Plot of average lactate concentration/voxel vs tumor volume. There is a suggestion of increased lactate with increasing volume. Lactate measurements are in mM/L

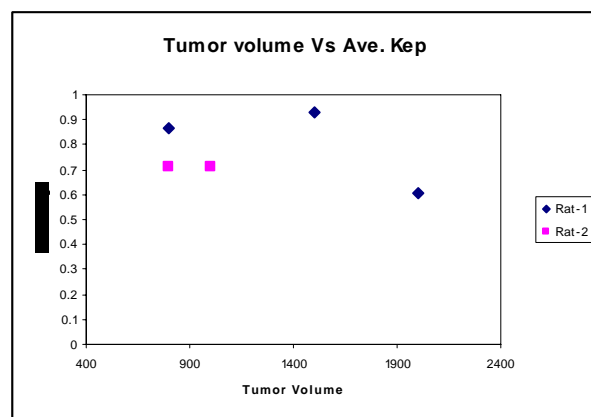


Fig. 8. Plot of average k_{ep} vs. tumor volume. Some data points were not obtained because of difficulty with catheterization